ESTUARINE NUTRIENT ANALYSES: A COMPARISON OF SAMPLE HANDLING TECHNIQUES AND THE ANALYSES OF CARBON, NITROGEN, PHOSPHORUS AND CHLOROPHYLL A

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ABSTRACT: An interlaboratory comparison involving filtering techniques as well as the results of nutrient analyses between four laboratories participating in the Chesapeake Bay Monitoring Program was conducted in March 1990. Samples from four distinctly different areas of the Chesapeake Bay were collected and then processed at one central location using each laboratory's routine filtering procedure. Sample analyses of the various components of carbon, nitrogen and phosphorus and chlorophyll a were then analyzed within 30 days using each laboratory's standard operating procedures.

Between laboratory agreement was generally good for nitrite, nitrite+nitrate, ammonium and phosphate. Where concentrations were at or near detection limits, the between and among laboratory variation was greatest.

The observed differences in filtering procedures between laboratories also apparently accounted for a portion of the differences in sample results. This is best demonstrated in the particulate carbon and particulate nitrogen data sets. Methodological differences for dissolved organic carbon contributed significantly to the observed differences for the higher salinity samples (<20 ppt). Between laboratory comparisons of total carbon, nitrogen and phosphorus were generally very good, as were the chlorophyll a values.

Results indicate that identical field sampling techniques between agencies could reduce some analytical variation. Establishment, early on, of an effective split sample QA program to identify and correct potential problems is crucial to any monitoring effort involving more than one agency.

ACKNOWLEDGEMENTS

Thank you to the personnel at VIMS for providing laboratory space and other facilities for our use in this exercise. Those individuals who participated in the study are also acknowledged. They are:

VIMS: K. Curling, G. Battisto, C. Adams, B. Neilsen and B. Salley.
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CBL: N. Kaumeyer, C. Keefe, A. Ward, and K. Wood

These individuals clearly take a personal as well as professional interest in providing quality data to the Chesapeake Bay Program.

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INTRODUCTION:

Data reliability and comparability between field/laboratory personnel from different institutions engaged in a monitoring program involving the same body of water are two prime concerns of managers and modelers. To improve accuracy and precision, D'Elia, et al. (1987) stressed the importance of direct measurement techniques for particulate analyses as well as more appropriate methods for the analyses of total dissolved nitrogen and phosphorus in estuarine/coastal waters. Standard EPA methods are often not sensitive enough to accurately analyze low level estuarine/coastal nutrient concentrations, so precision and comparability between institutions are often reduced. Clearly, the most precise and accurate data practically obtainable are required to obtain adequate scientiific information to make sound management decisions.

Largely as a result of that study, the Chesapeake Bay Program (CBP), in 1988 directed the three laboratories involved in the Chesapeake Bay Mainstem Monitoring Program to use direct methods for the analyses of particulate carbon, nitrogen and phosphorus and also to use methods for the analyses of total dissolved N and P which are more consistent with the oceanographic community. Those three laboratories are the University of Maryland System Chesapeake Biological Laboratory (CBL), College of William and Mary's Virginia Institute of Marine Science (VIMS) and Old Dominion University's Applied Marine Research Laboratory (ODU).

With similar methods in place, a split sample quality assurance (QA) program was initiated in 1988. This program has gone through some modifications in sampling design but during the period reported in this study Maryland Dept. of the Environment (MDE) field crews processed and distributed samples to CBL while a VIMS field crew processed and distributed samples to the two Virginia laboratories. Initial results from this QA program indicated some discrepancies in the sample results between the laboratories (Bergstrom, 1990) but the report could not ascertain the reasons for these differences.

It was decided by personnel from each of the laboratories to conduct an intercalibration exercise. This exercise was held at VIMS on 21 March 1990. The purpose was to bring all three laboratories together to process previously collected water samples - representative of concentrations normally found in the Chesapeake Bay. Actual analyses of these samples were to be carried out at the various laboratories. By processing batch samples, all variation normally found associated with shipboard collection procedures would be eliminated.

MATERIALS AND METHODS

WATER: Four distinctly different water samples were used in this exercise.

Sample A: This sample was a mixture of estuarine and ocean water which was collected at the mouth of the Chesapeake Bay. The salinity of the sample was 26.6 parts per thousand (ppt). The following nutrient concentrations were added:

Ammonium	0.1 mg N/L
Nitrite	0.05 mg N/L
Nitrate	0.1 mg N/L
Phosphate	0.05 mg P/L
Carbon	3.0 mg C/L
Urea	0.3 mg N/L

Sample B: This sample consisted of a mixture of estuarine and ocean water which was collected from the same location as sample A (26.6 ppt) but contained ambient dissolved and particulate nutrient concentrations.

Sample C: Sample C was a low salinity sample (3.1 ppt) which contained relatively high concentrations of dissolved and particulate nutrients. This sample was collected from the upper Chesapeake Bay.

Sample D: Sample D was collected from the James River. It contained a salinity of 7.9 ppt and was generally low in dissolved and particulate nutrient concentrations.

These samples were collected up to two days before the exercise was performed. All samples were pumped into large (20 gallon) carboys and refrigerated until 22 March 1990.

Each sample was then carefully poured into a 30 gallon pre-rinsed plastic garbage pail. A spigot had been installed in the bottom of each garbage pail 24 hours prior. This spigot facilitated easy sample collection.

The samples were manually stirred using a wooden oar to prevent particle settling. Each laboratory then collected seven replicate sub samples from each sample in the following manner.

Each laboratory alternately collected the seven replicates water samples from one garbage pail using sample-rinsed two liter poly bottles. After all the laboratories had collected and processed the replicates from one sample, the procedure was repeated with the next sample. Thus, each sample was processed by the three laboratories at the same time.

Participants then processed their set of samples according to their normal field protocol. Each laboratory used subtle differences in their sample processing techniques. A summary of each follows.

Field Sampling Protocols:

CBL: Figure 1 illustrates the manifold system normally used in the Mainstem Monitoring Program. Please refer to this figure during the following description.

Total Suspended Solids (TSS)/Particulate Phosphorus (PP): A known volume of sample is filtered through prenumbered and preweighed 47 mm GF/F filter pads. The pads are then rinsed with 2 - 5 ml. aliquots of deionized (DI) water while still on the filtration apparatus. Pads are then folded in half using forceps, placed side by side in a labelled aluminum foil pouch and then frozen for later analysis. Filtration is performed in replicate and the filtrate is discarded because of its alteration by the DI water rinse.

Particulate Carbon (PC)/Particulate Nitrogen (PN): A known volume of sample is filtered through precombusted 25 mm GF/F filter pads. After filtration, the pads are folded in half using forceps and placed side by side in a labelled aluminum foil pouch and then frozen for later analysis. Filtration is done in replicate and the filtrate is combined and used for the analyses of the dissolved components.

Four, four ml AutoAnalyzer cups and caps are sample rinsed three times and then filled 2/3 full with sample and capped. Three are frozen, two of which are used in the ammonium, nitrite, nitrite+nitrate and phosphate analyses. The third is used as a back-up. The fourth cup is refrigerated and used for the silicate analysis.

Ten milliliters of filtrate are also added to a screw cap test tube after sample rinsing and then frozen. This sample is analyzed for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP).

A sample is also collected for dissolved organic carbon (DOC) by sample rinsing a 30 mL screw cap glass bottle, filling 2/3 full with the filtrate and then freezing.

Chlorophyll a: The two 25 mm filtration sets are reused for the chlorophyll sample. A known volume of sample is filtered through 25 mm GF/F filter pads. After filtration, the pads are folded in half using forceps, placed side by side in a labelled aluminum foil pouch, and then frozen for later analysis. Pads are not rinsed with magnesium carbonate; therefore, the filtrate could be used, if necessary, for dissolved fraction analyses. Chlorophyll samples are analyzed by the State of Maryland's Dept. of Health and Mental Hygiene Laboratory in Baltimore, MD. (DHMH). The filters are ground and allowed to extract in approximately 10 ml of 90% acteone in a refrigerator over night.

VIMS: Figure 2 illustrates the manifold system normally used in their portion of the Mainstem Monitoring Program. Please refer to this figure during the following description.

TSS/PP: A known volume of sample is filtered through prenumbered and preweighed 47 mm GF/F filter pads. Filtrate from one flask is placed in poly bottles for later analysis of ammonium, phosphate, nitrite, nitrite+nitrate, TDN and TDP and then frozen. Filtrate from the other filter flask is placed in a separate poly bottle for silicate analysis. Two, five ml aliquots of deionized water are then used to rinse the TSS/PP pads. The pads are then placed in petri dishes, wrapped in aluminum foil, labelled and frozen for later analysis.

PC/PN: A known volume of sample is filtered through precombusted Gelman 13 mm AE glass fibre filters. After filtration the pads are placed in labelled petri dishes and frozen for later analyses. Three to four pads are normally collected per sample. Two pads are analyzed together, thus comprising one sample. The final concentration is calculated by summing the volumes filtered.

The filtrate which is collected by this method is used for the analysis of DOC as well as the other dissolved constituents.

Chlorophyll a: A known volume of sample is filtered through 47 mm GF/F filter pads which have been pre-rinsed with magnesium carbonate. The pads are then placed in petri dishes, wrapped in aluminum foil, labelled and frozen for later analysis.

ODU: Figure 3 illustrates the manifold system normally used in their

Figure 1. Filtration scheme of Chesapeake Biological Laboratory. Water was filtered separately for chlorophyll-A using the 25 mm filtration apparatus.

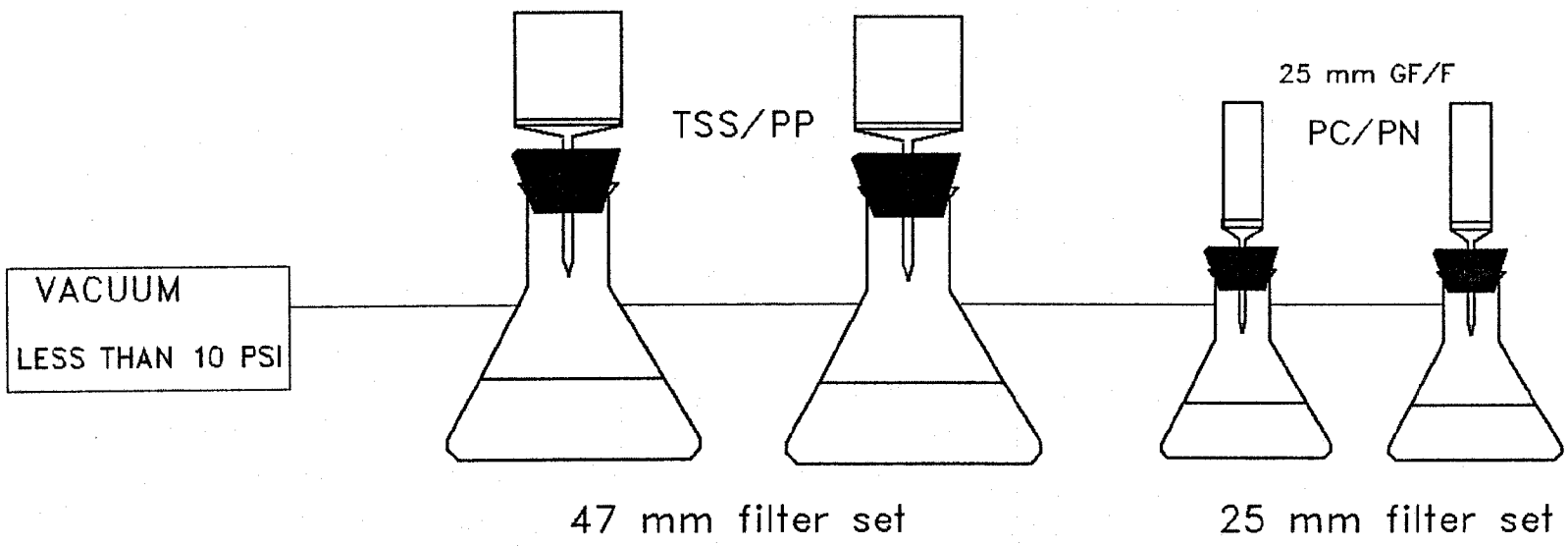
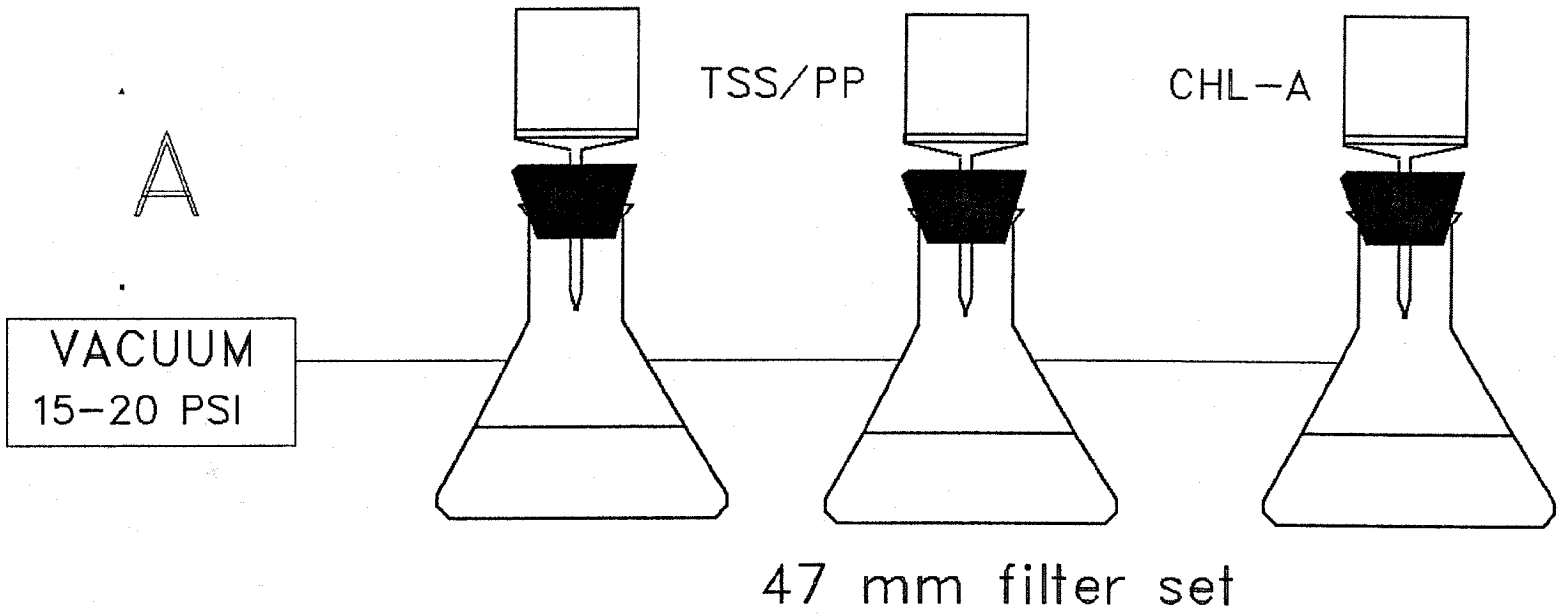

VACUUM
LESS THAN 10 PSI
TSS/PP
25 mm GF/F
PC/PN
47 mm filter set
25 mm filter set

Figure 2. Filtration scheme of Virginia Institute of Marine Science. Dissolved constituents collected from the PC/PN filtrate (B).


A
TSS/PP
CHL-A
VACUUM
15-20 PSI
47 mm filter set

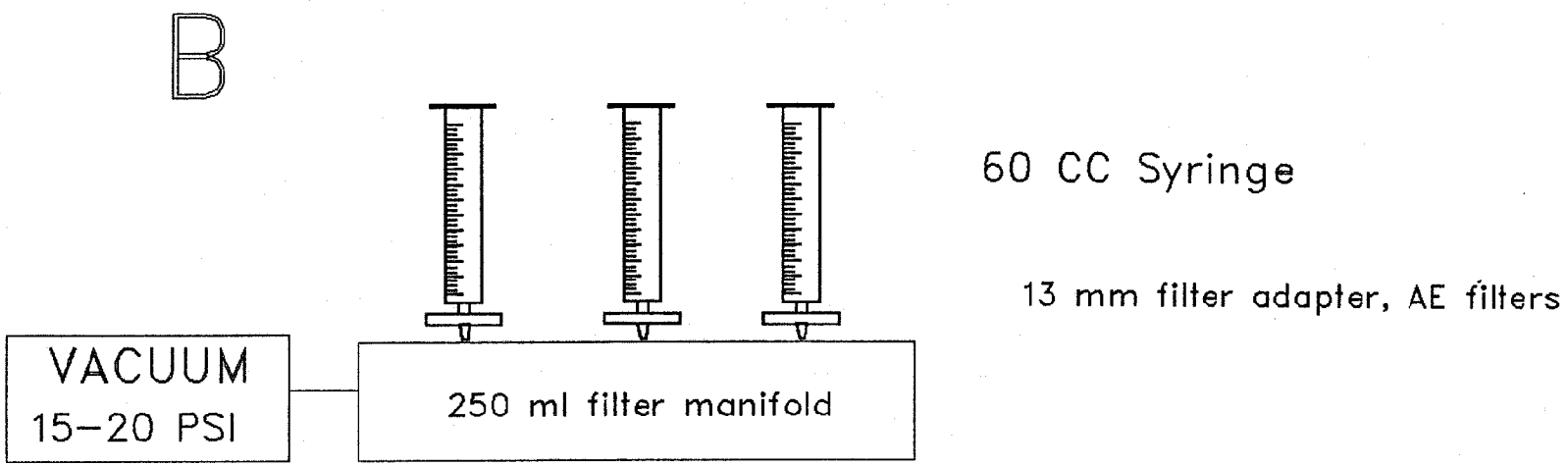
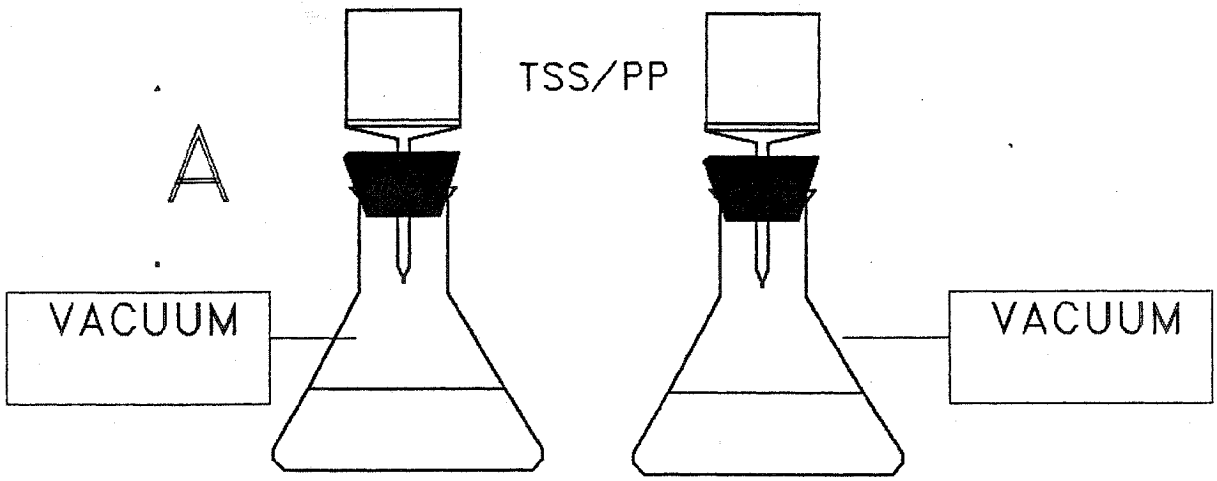

B
60 CC Syringe
13 mm filter adapter, AE filters
VACUUM
15-20 PSI
250 ml filter manifold

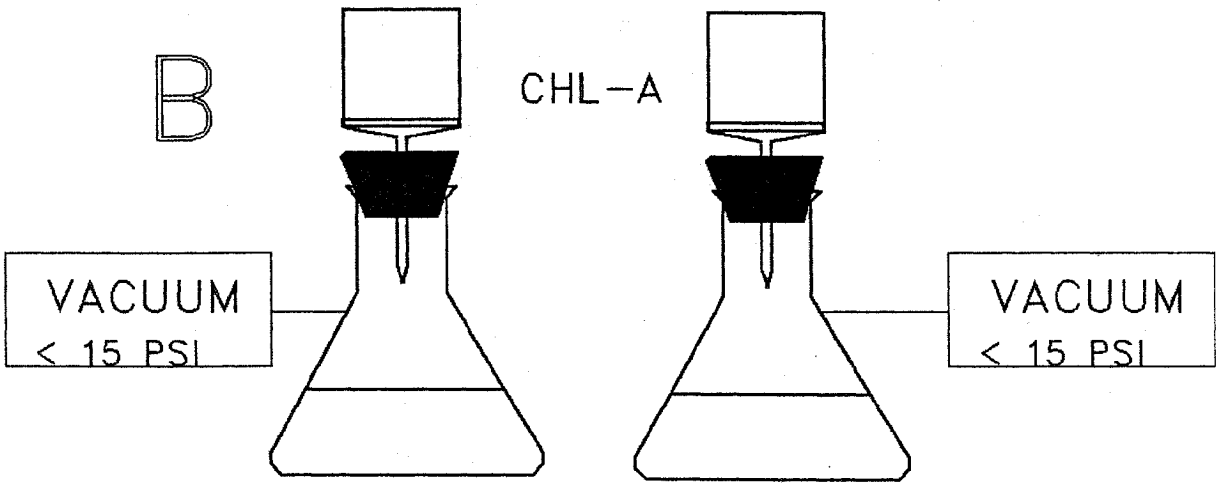
Figure 3. Filtration scheme of Old Dominion University.

Separate vacuum pump used for each filtration set (A & B).

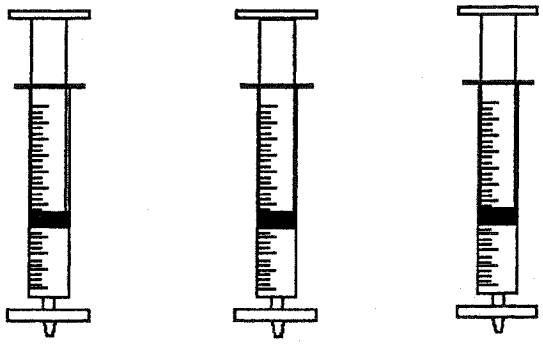
Samples for PC/PN pressure filtered (C).



47 mm filter set



47 mm filter set



60 CC Syringe
Pressure filtered through

13 mm filter adapter, AE filters

Filtrate collected for DOC analysis

portion of the Mainstem Monitoring Program. Please refer to this figure during the following description.

TSS/PP: A known volume of sample is filtered through prenumbered and preweighed 47 mm GF/F filter pads. Before the pads are rinsed with 2 - 5 ml. aliquots of deionized water, the filtrate is removed and used for analysis of the dissolved fraction (ammonium, nitrite, nitrite+nitrate, phosphate, TDN, TDP). This filtrate is placed in acid washed poly bottles and frozen for later analyses. Silicate samples are placed in separate poly bottles and refrigerated. The filter pads are placed in pre-numbered snap on plastic containers.

PC/PN: A known volume of sample is pressure filtered through precombusted Gelman 13 mm AE glass fiber filters using hand held syringes adapted with 13 mm Swinlok filter holders. Four pads per sample are normally collected. After filtration, the pads are folded in quarters and placed in acid cleaned glass vials (one pad/vial) and frozen for later analysis. Two pads are analyzed per sample with the final concentration calculated by summing the actual volume filtered.

Filtrate from this fraction is placed in poly bottles, and frozen for analyses of DOC.

Chlorophyll-A: Magnesium carbonate is added to a known volume of sample and filtered through 47 mm GF/F pads. The pads are then immediately ground using a tissue grinder, placed in less than 8 mL of 90% acetone, and kept dark in a refrigerator until analysis.

ANALYTICAL METHODS:

A synopsis of each of the laboratory's analytical methods is provided in Tables 1-3. A complete description of the methods can be obtained from each of the laboratories.

RESULTS AND DISCUSSION

INORGANIC NUTRIENTS

Ammonium: Ammonium concentrations for Sample B were below the detection limits of CBL (0.003 mg N/L) and ODU (0.0056 mg N/L, Figure 4). For samples A, C and D there was excellent agreement between the laboratories. Concentrations of 0.085, 0.107, and 0.089 mg N/L were reported for sample A, 0.147, 0.131 and 0.124 mg N/L for sample C for CBL, VIMS and ODU, respectively. All laboratories reported a mean concentration of 0.012 mg N/L for sample D. Coefficients of variation in most cases were less than 5% for all laboratories.

Nitrite+Nitrate: Results of the nitrite+nitrate analysis are found in Figure 5. Results from sample A, C and D showed excellent agreement between the participating laboratories. Results of all three samples ranged between 92 and 108% of the mean determined by the three laboratories. Coefficients of variation were also extremely low (<5.0%). Concentrations of 0.0053, 0.0020, and 0.0025 mg N/L were reported for sample B by CBL, VIMS and ODU, respectively. Coefficients of variation for these sample results were all approximately 20%. The concentration reported by CBL was much higher than the other two laboratories.

Table 1. ANALYTICAL METHODS - DISSOLVED INORGANIC ANALYSES

Analyte: Ortho-phosphate

LABORATORY	METHOD	INSTRUMENT	SPECIFIC COMMENTS
CBL	Automated, double reagent	AutoAnalyzer II	Murphy and Riley, 1965. Ascorbic Acid as separate reagent
VIMS	Automated, single reagent	AutoAnalyzer II	Murphy and Riley, 1965.
ODU	Manual, double ragent	Spectrophotometer	Murphy and Riley, 1965.

Analyte: Nitrate+Nitrite

CBL	Automated Cadmium Reduction	AutoAnalyzer II	50 mm flowcell, 550 nm filter
VIMS	Automated Cadmium Reduction	AutoAnalyzer II	50 mm flowcell, 550 nm filter
ODU	Automated Cadmium Reduction	AutoAnalyzer II	75 mm flowcell, 550 nm filter

Analyte: Nitrite

CBL	Automated Sulfanilamide	TrAAcs-800	50 mm flowcell, 520 nm filter
VIMS	Automated Sulfanilamide	AutoAnalyzer II	50 mm flowcell, 550 nm filter
ODU	Automated Sulfanilamide	AutoAnalyzer II	75 mm flowcell, 550 nm filter

Analyte: Ammonium

CBL	Automated Phenol hypochlorite	TrAAcs-800	50 mm flowcell, 630 nm filter
VIMS	Automated Phenol hypochlorite	AutoAnalyzer II	50 mm flowcell, 650-660 nm filter
ODU	Automated Phenol hypochlorite	AutoAnalyzer II	75 mm flowcell, 630 nm filter

Table 2. ANALYTICAL METHODS - TOTAL DISSOLVED

Analyte: Total Dissolved Phosphorus (TDP)

LABORATORY	METHOD	INSTRUMENT	SPECIFIC COMMENTS
CBL	Alkaline Persulfate Digestion	AutoAnalyzer II	D'Elia, et al., 1977
VIMS	Alkaline Persulfate Digestion	AutoAnalyzer II	D'Elia, et al., 1977
ODU	Acid Persulfate Digestion	Spectrophotometer	Menzel and Corwin, 1965

Analyte: Total Dissolved Nitrogen

CBL	Alkaline Persulfate Digestion	AutoAnalyzer II	D'Elia, et al., 1977
VIMS	Alkaline Persulfate Digestion	AutoAnalyzer II	D'Elia, et al., 1977
ODU	Alkaline Persulfate Digestion	AutoAnalyzer II	D'Elia, et al., 1977

ANALYTICAL METHODS - DISSOLVED ORGANIC

Analyte: Dissolved Organic Carbon

CBL	Persulfate oxidation at 110^{o} C	OI Model 700 Carbon Analyzer	Menzel and Vaccaro, 1964
VIMS	High temp. (680^{o}C) combustion	Shimadzu Model 500	Sugimura and Suzuki, 1988
ODU	Persulfate oxidation at 110^{o} C	OI Model 524 Carbon Analyzer	Menzel and Vaccaro, 1964

Table 3. ANALYTICAL METHODS - PARTICULATE ANALYSES

Analyte: Chlorophyll-A

LABORATORY	METHOD	INSTRUMENT	SPECIFIC COMMENTS
DHMH	Spectrophotometric	Beckman Dual Beam	Sample frozen, thawed, ground, extracted, analyzed
VIMS	Spectrophotometric	Milton Roy Scanning	Sample frozen, thawed, ground, extracted, analyzed
ODU	Spectrophotometric	Perkin Elmer Dual Beam	Sample ground, extracted and analyzed

Analyte: Particulate Carbon/ Particulate Nitrogen (PC/PN)

CBL	High Temp. Combustion	Control Equip. Model 240 XA	Regulated vacuum filtration system, 25 mm GF/F filters, one pad analyzed per sample
VIMS	High Temp. Combustion	Carlo Erba NA1500	Regulated vacuum filtration through 60 cc syringes with 13 mm swin lok holder 13 mm AE filters used, multiple filters to get one answer
ODU	High Temp. Combustion	Carlo Erba NA1500	Unregulated pressure filtration through 60 cc syringes with 13 mm swin lok holder, 13 mm AE filters used, multiple filters to get one answer

Analyte: Particulate Phosphorus (PP)

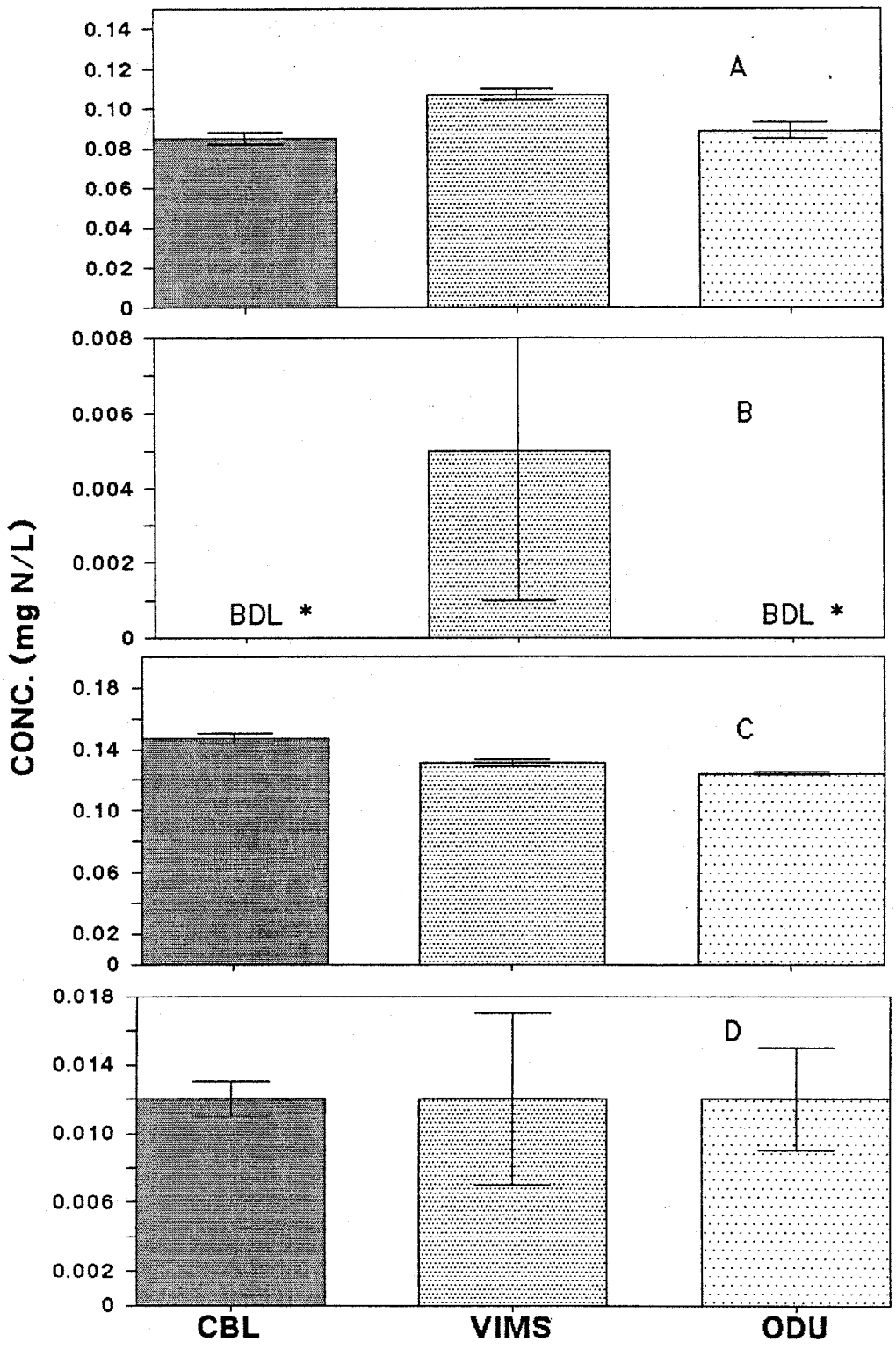
CBL	High Temp. Combustion, HCl extraction	Automated Phosphate	Aspila, et al., 1976
VIMS	High Temp. Combustion, HCl extraction	Automated Phosphate	Aspila, et al., 1976
ODU	High Temp. Combustion, HCl extraction	Manual Phosphate	Aspila, et al., 1976

Analyte: Total Suspended Solids (TSS)

CBL	Weight, by difference	Manual/Weight Processing	Standard Methods,
VIMS	Weight, by difference	Manual	Standard Methods,
ODU	Weight, by difference	Manual	Standard Methods,

Figure 4. Concentrations of ammonium for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS) and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

AMMONIUM



* BDL = BELOW DETECTION LIMIT

Figure 5. Concentrations of nitrite+nitrate for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science, and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

NITRITE + NITRATE

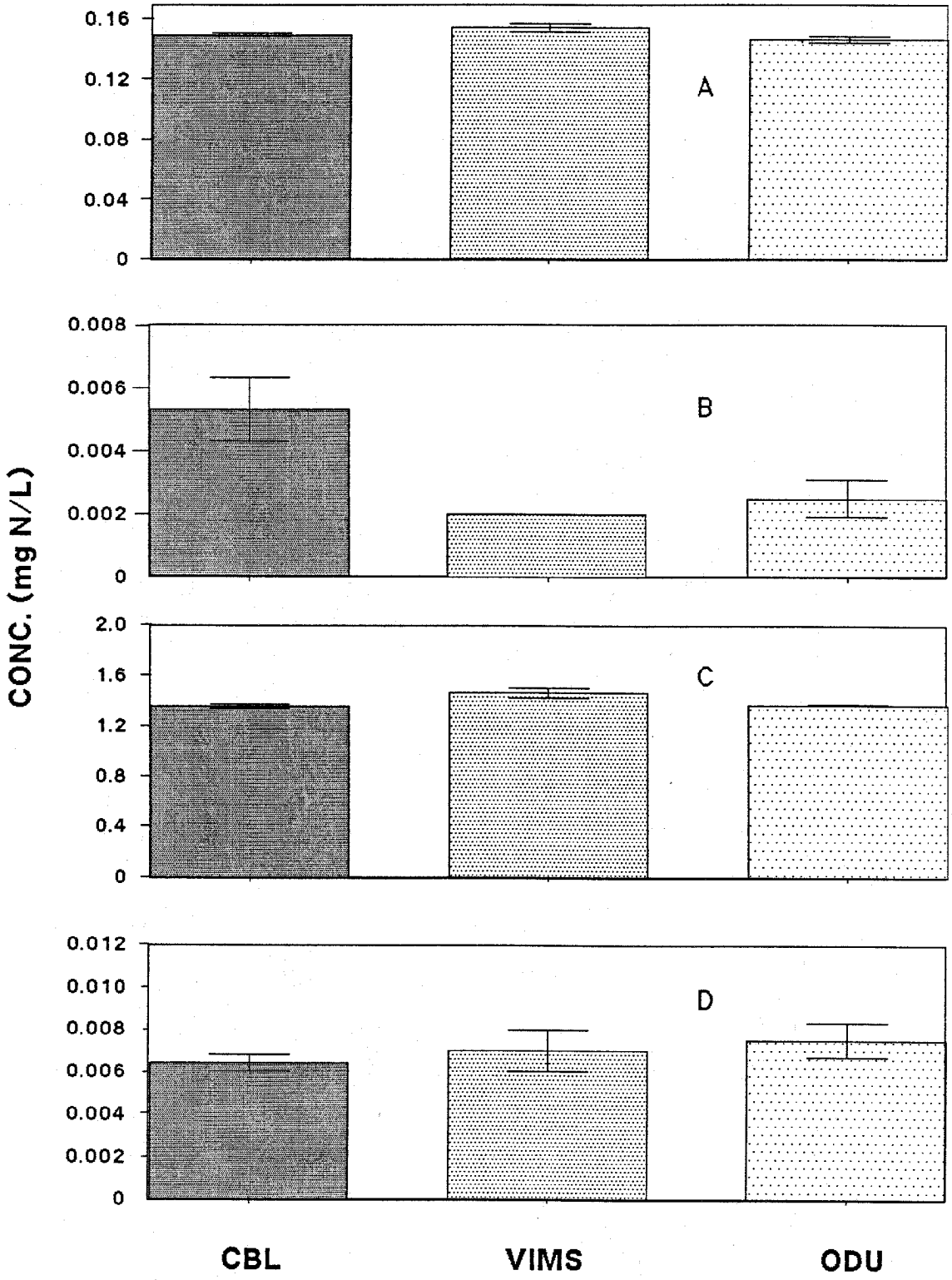
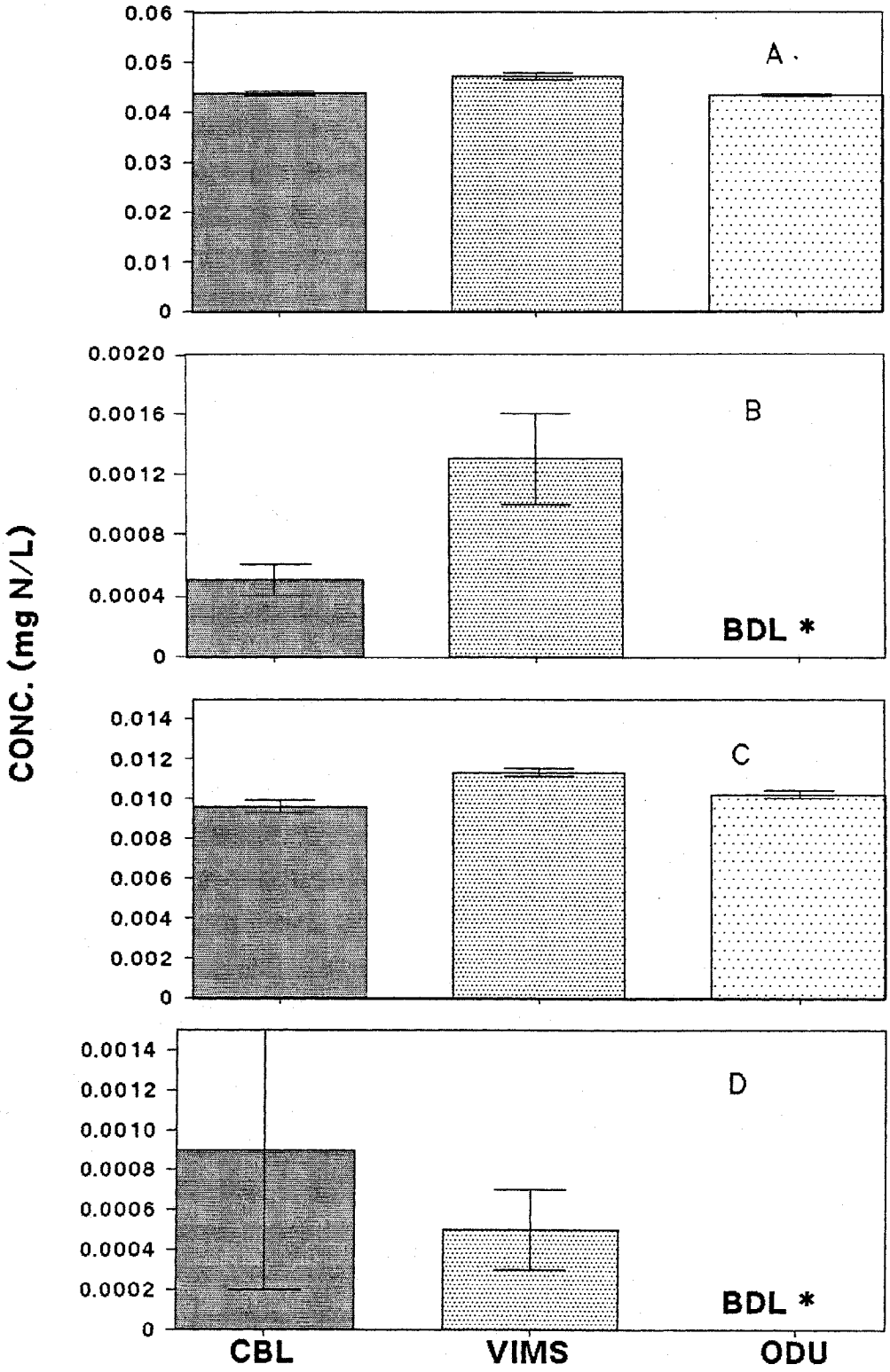


Figure 6. Concentrations of nitrite for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

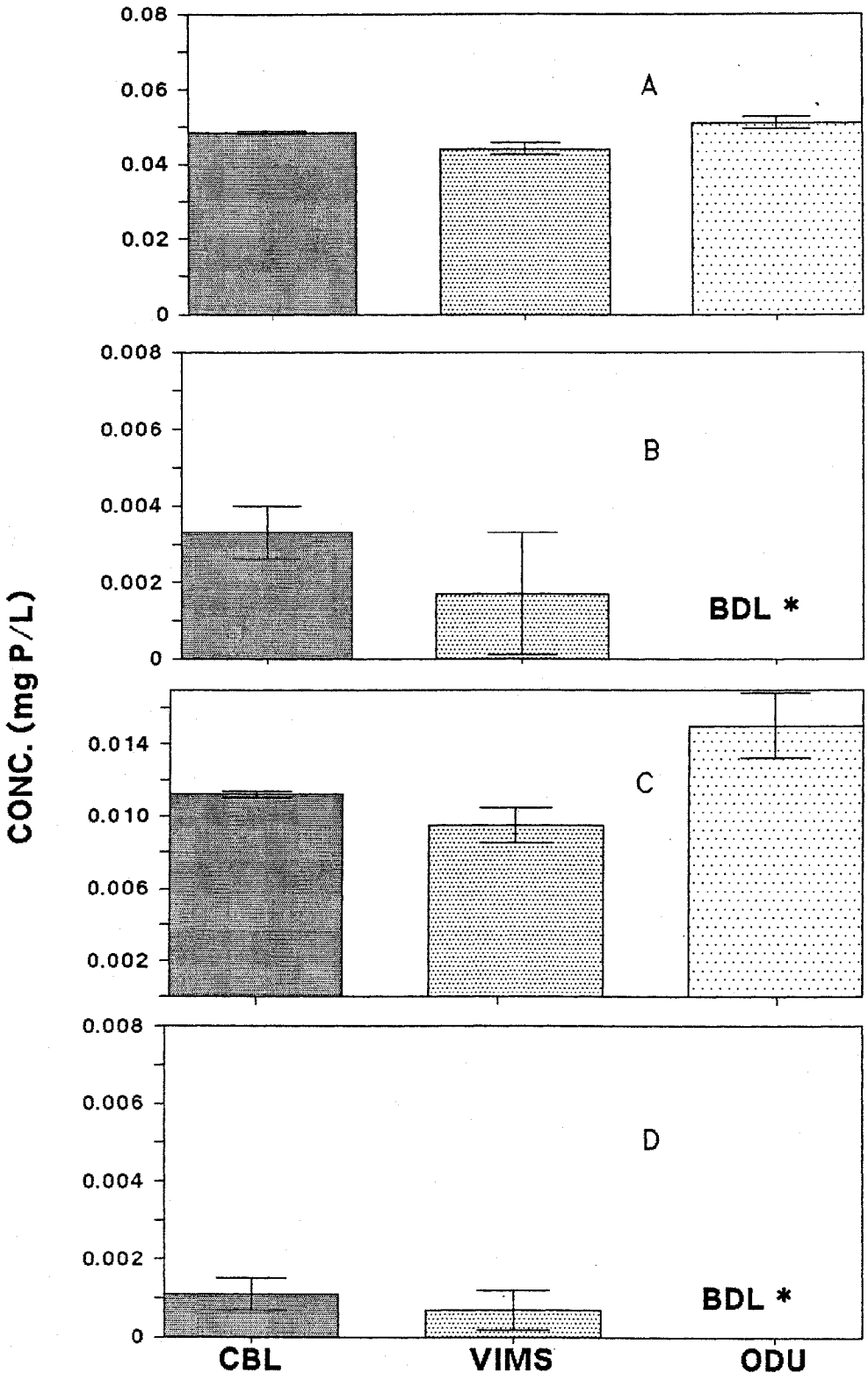
NITRITE



* BDL = BELOW DETECTION LIMIT

Figure 7. Concentrations of ortho-phosphate for Samples A-D analyzed by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion Universiity (ODU). Concentration units are mg/L and error bars represent one standard deviation.

ORTHO-PHOSPHATE



* BDL = BELOW DETECTION LIMIT

Nitrite: Figure 6 represents the results of the nitrite analysis. Results from sample A and C were all within 10% of the mean concentration of the three laboratories. The actual reported concentrations were: 0.0439, 0.0472 and 0.0433 mg N/L for sample A and 0.0096, 0.0113, and 0.0102 mg N/L for sample C for CBL, VIMS and ODU, respectively.

Results from ODU for Samples B and D were not reported because they were below the detection limit for nitrite (0.001 mg N/L). Poor replication of the reported mean values of 0.0005 mg N/L (CBL) and 0.0013 mg N/L (VIMS) for sample B were also noted. Coefficients of variation for the two laboratories for this sample were both approximately 20%. Mean concentrations of 0.0009 and 0.0005 mg N/L were reported for sample D by CBL and VIMS, respectively. Again, coefficients of variation for this sample were extremely high (~80%). When concentrations approach detection limits, the variation is greatly increased.

Ortho phosphate: Results are found in Figure 7. Results from sample A showed excellent agreement between the three laboratories. All concentrations fell between 92 and 107% of the mean. Concentrations of 0.0484, 0.0441, and 0.0510 mg P/L were reported by CBL, VIMS and ODU, respectively. Fairly good agreement was obtained for sample C between the three laboratories. Mean concentrations of 0.0112, 0.0095 and 0.0150 mg P/L with corresponding coefficients of variation of 2, 11 and 12% were determined by CBL, VIMS and ODU, respectively.

Results from ODU for Samples B and D were not reported because they were below the detection limit of that laboratory (0.005 mg P/L). Results of 0.0033 and 0.0017 mg P/L were reported for sample B by CBL and VIMS. Much of the differences noted in this sample can be attributed to within sample reproduceability. Coefficients of variation for this sample were 21% for CBL and 94% for VIMS. This poor replication for low level samples was also encountered for sample D, although the mean concentration (0.0009 mg P/L) was approximately 80% of each of the laboratories reported concentrations.

TOTAL DISSOLVED COMPONENTS: Total dissolved nitrogen and phosphorus refer to the dissolved inorganic + organic component of the dissolved sample.

Total Dissolved Nitrogen: Results of the total dissolved nitrogen (TDN) analyses are found in Figure 8. TDN concentrations for sample A and B were quite similar for CBL and VIMS (0.720 and 0.764; 0.180 and 0.227 mg N/L, respectively). ODU concentrations for these same samples were lower (0.578 and 0.138 mg N/L, respectively).

The mean concentrations from sample C were all within a few percent of each other (1.66, 1.55, and 1.56 mg N/L) for CBL, VIMS and ODU, respectively; while the concentration for sample D reported by ODU was approximately 30% lower than that of CBL, which was 28% lower than VIMS (0.166 mg N/L).

Total Dissolved Phosphorus: Where concentrations were greater than 0.01 mg P/L (samples A and C) agreement between the three laboratories was very good (Figure 9).

In instances where the concentration was less than 0.01 mg P/L, variation between replicates of each lab, as well as between laboratory variation was

Figure 8. Concentrations of total dissolved nitrogen for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

TOTAL DISSOLVED NITROGEN

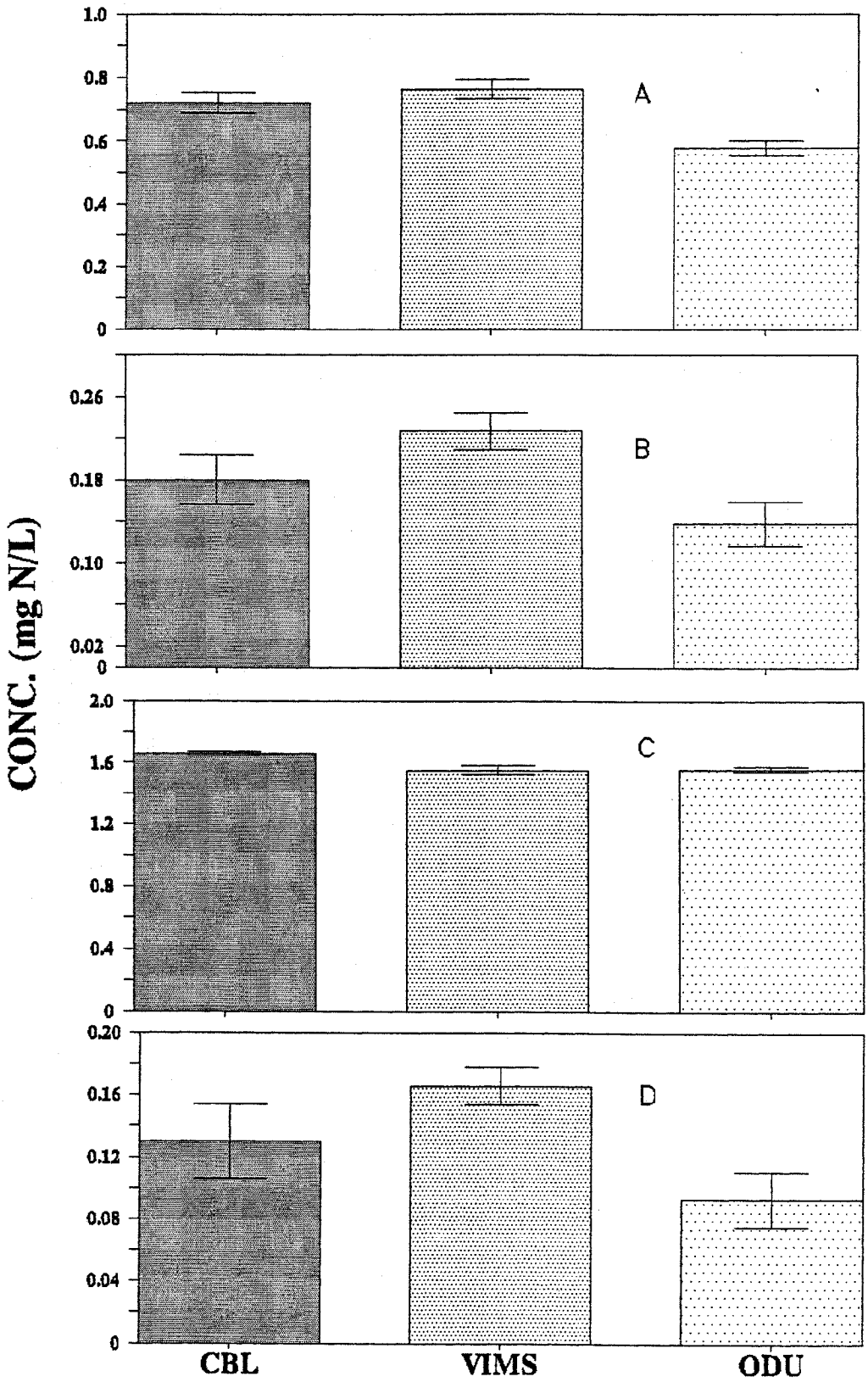
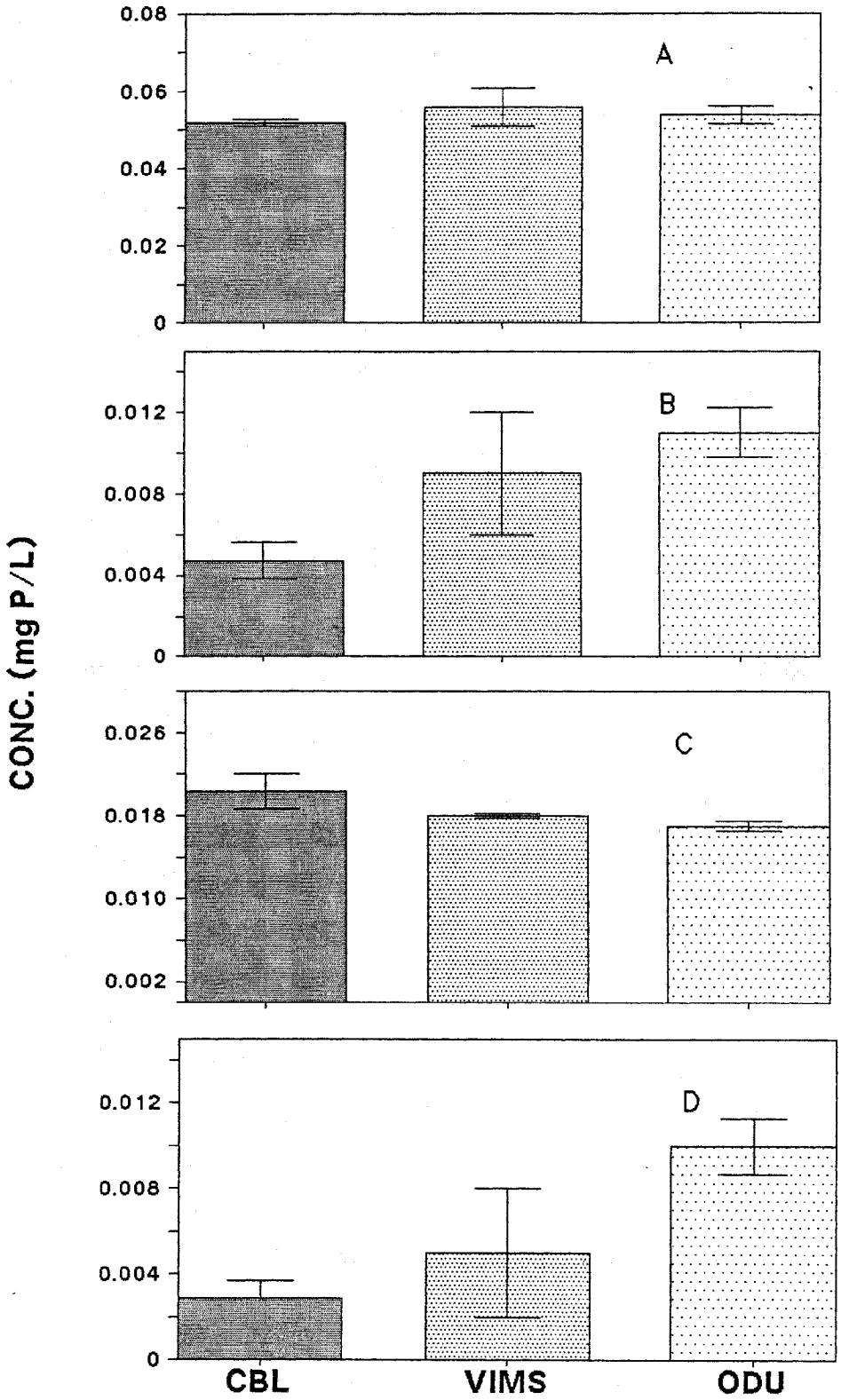


Figure 9. Concentrations of total dissolved phosphorus for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

TOTAL DISSOLVED PHOSPHORUS



greatly increased. For sample B, concentrations ranged from 0.005 to 0.011 mg P/L with coefficients of variation of 11-33%. Concentrations of sample D were also quite variable, ranging from 0.003 to 0.01 mg P/L and coefficients of variation ranging from 13 to 60%.

ORGANIC NUTRIENTS

Dissolved Organic Carbon (DOC): Differences in sample results between laboratories are particularly evident in Samples A, B, and C (Figure 10) . These variations are primarily due to methodological/instrumentation differences among the laboratories.

DOC concentrations reported by VIMS were obtained from a Shimadzu Model 500 Carbon Analyzer. The analytical method utilized is a high temperature catalytic oxidation technique (680 degrees C), followed by the determination of the amount of CO_2 generated by infra red gas analysis. It should be noted that the aluminum oxide catalyst is impregnated with 3% platinum (Sugimura and Suzuki, 1988).

A persulfate digestion technique (Menzel and Vacarro, 1964) at 100 degrees C was utilized by the other two laboratories. ODU used an OI Model 524 Carbon Analyzer which is an ampulated method where the sample, phosphoric acid and persulfate are placed in an ampule, sealed and autoclaved. The resultant CO_2 gas is measured by near infra red. An OI Model 700 Carbon Analyzer is used by CBL. This is a more automated method where the sample, phosphoric acid and persulfate are added together in an internal digestion vessel. The CO_2 gas produced is sent to a near infra red source for analysis. The theory of these two instruments is the same but the Model 700 is much less labor intensive.

Recent studies (Sugimura and Suzuki, 1988; Suzuki, et al., 1990; Sharp, et al., 1988) all measure consistently higher concentrations of DOC in open ocean and coastal waters using the high temperature catalytic technique over measurements by the persulfate digestion technique. In estuarine waters, results of the same sample using the two techniques converge to little or no difference in the salinity range of 12- 15 parts per thousand (ppt) (Sharp, et al., 1988; Salley, unpublished).

These results are consistent with those shown in Figure 10. Sample A (salinity: 26.6 ppt) analyzed by VIMS high temperature catalytic technique resulted in consistently higher values than when analyzed by ODU using the ampulated persulfate method. CBL values were considerably lower than the other two laboratories. As the salinity decreased, concentrations of DOC determined by the three laboratories were more consistent with each other (Table 4).

Figure 10. Concentrations of dissolved organic carbon for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

DISSOLVED ORGANIC CARBON

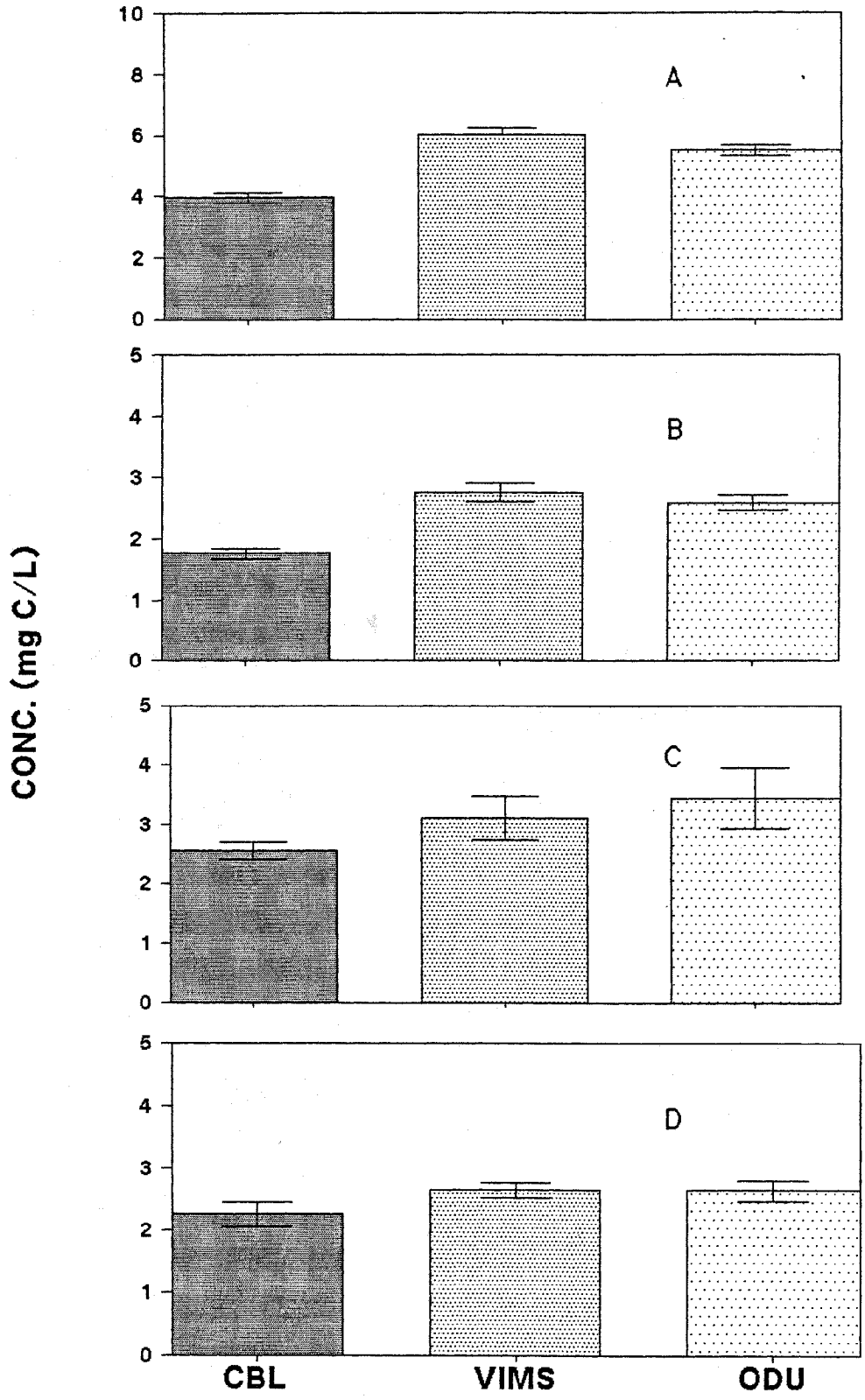


Table 4. Dissolved Organic Carbon results from laboratories using different techniques and instrumentation.

Sample	Salinity (ppt)	Laboratory	Mean (mg C/L)	Std. Dev. (mg C/L)
A	26.6	CBL	3.96	0.16
		VIMS	6.01	0.21
		ODU	5.52	0.19
B	26.6	CBL	1.76	0.08
		VIMS	2.76	0.15
		ODU	2.60	0.13
C	3.1	CBL	2.56	0.14
		VIMS	3.11	0.37
		ODU	3.43	0.51
D	7.9	CBL	2.26	0.20
		VIMS	2.65	0.12
		ODU	2.64	0.17

From these data it appears that the Shimadzu Carbon Analyzer (VIMS) recovered the greatest amount of C, closely followed by the ampulated persulfate technique (ODU) with the automated persulfate technique (CBL) recovering the least. Results from an on-going study (Salley, unpublished) also support these results.

A possible explanation for these observed differences between the two techniques in more saline waters may be in the ability of the methods to break down various sized molecular weight groups. Sugimura and Suzuki (1988) found that the high temperature catalytic method was able to break down a total of 239 molecular weight groups compared to 87 for the persulfate technique. The persulfate technique will recover only a small percentage of molecular weight groups in the 0.18 to 10 X 10^4 daltons and even higher, whereas the high temperature catalytic method recovers a much greater percentage of these groups. This is particularly evident in ocean and coastal samples and most probably explains these differences between results of each of the laboratories. Apparently, fresh water does not contain a significant portion of these compounds, hence recoveries of C by the two methods are quite similar in the less saline samples.

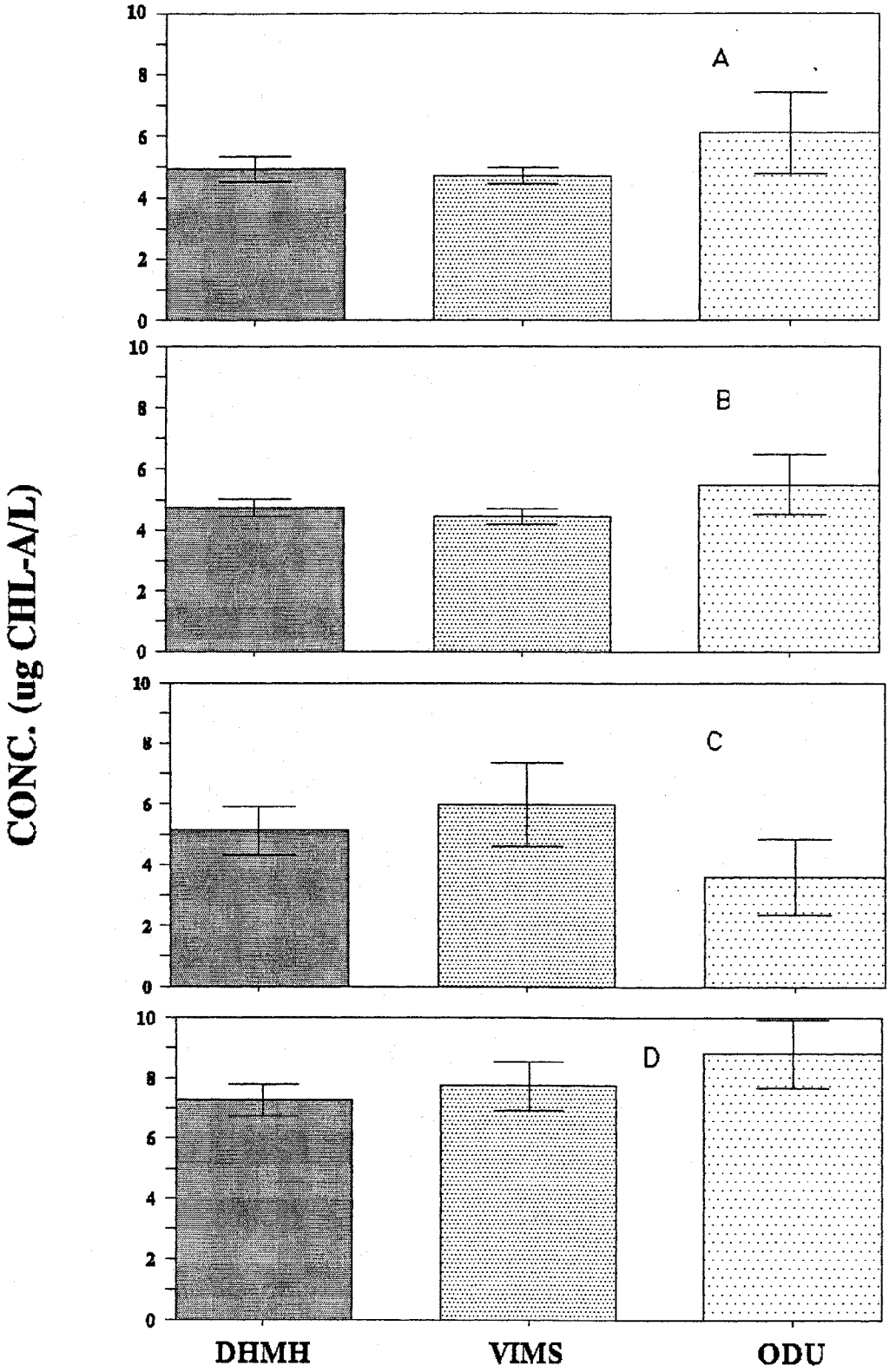
PARTICULATE COMPONENTS

Chlorophyll a:

ODU reported slightly higher concentrations than DHMH or VIMS for samples A, B and D. The standard deviations and corresponding coefficients of variation were also larger (Figure 11). DHMH and VIMS concentrations were quite similar for these samples, with a difference of only a few percent between mean values. Operationally, ODU immediately ground their filters and placed them in acetone, while the other two laboaratories froze the filters for later extraction. This may be a cause of a part of the observed variation.

Figure 11. Concentrations of chlorophyll-A for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are ug/L and error bars represent one standard deviation.

CHLOROPHYLL-A



Sample C concentrations for ODU were lower (3.6 +/- 1.26 ug/L) than either DHMH (5.12 +/- 0.8 ug/L) or VIMS (5.98 +/- 1.38 ug/L) but the relatively large coefficients variation for this sample (ODU, 35%; DHMH, 16%, and VIMS, 23%) indicate little difference between these results.

Particulate Phosphorus/Total Suspended Solids: The same filter pads are used for both of these analyses and consequently will be discussed in one section. After the differences in weights have been determined for total suspended solids (TSS), the pads are then processed for Particulate P (PP).

Particulate phosphorus results are presented in Figure 12. Samples A and B were essentially replicate samples because no "particulate spikes" were added. Mean percent differences of these two samples within each laboratory were excellent (0 - 4%). Mean results of these two samples were comparable between CBL and ODU (0.0163 and 0.0170 mg P/L, respectively) while VIMS concentrations were consistently lower (0.0129 mg P/L). Coefficients of variation ranged from 2 - 15%.

Agreement between laboratories was generally excellent for samples C and D (Figure 12) with coefficients of variation in the same range as samples A and B.

TSS results are presented in Figure 13. Where PP values were lower in Samples A and B for VIMS, the opposite is true for TSS. VIMS mean concentration for these two samples was 19.1 mg/L, while CBL and ODU reported 13.9 and 14.5 mg/L, respectively. These higher concentrations may be due to inadequate rinsing of the pads with deionized water. This step is crucial to remove salt from the pads, but does not explain why the PP concentrations were so much lower than the other two labs.

Sample C, which was considered a high particulate sample, showed little variation in results between the three laboratories (CBL: 75.4, VIMS: 73.6, and ODU: 71.1 mg/L) while VIMS results for sample D were slightly greater than the other two labs (CBL: 8.6, VIMS: 11.9, and ODU: 8.8 mg/L).

Particulate Carbon/Particulate Nitrogen (PC/PN):

Results of the PC/PN analyses are presented Figures 14 and 15.

Particulate C: Coefficients of variation from all laboratory results for the four samples ranged from 4 - 14%. Between sample agreement of samples A and B for each laboratory was 97, 98 and 96 % for CBL, VIMS and ODU, respectivley. Mean concentrations for each sample were as follows: Sample A and B: 1.05 mg C/L; Sample C: 3.4 mg C/L; and Sample D: 0.84 mg C/L.

Particulate N: Coefficients of variation determined from all laboratory values for the four samples ranged from 3 - 19%. Between sample reproduceability for samples A and B were excellent; 99, 109 and 102% for CBL, VIMS and ODU, respectively. Pooled, mean concentrations were 0.122, 0.119, 0.322 and 0.103 mg N/L for samples A-D, respectively.

Consistently higher concentrations for PC/PN were reported by CBL than either VIMS or ODU.

Figure 12. Concentrations of particulate phosphorus for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

PARTICULATE PHOSPHORUS

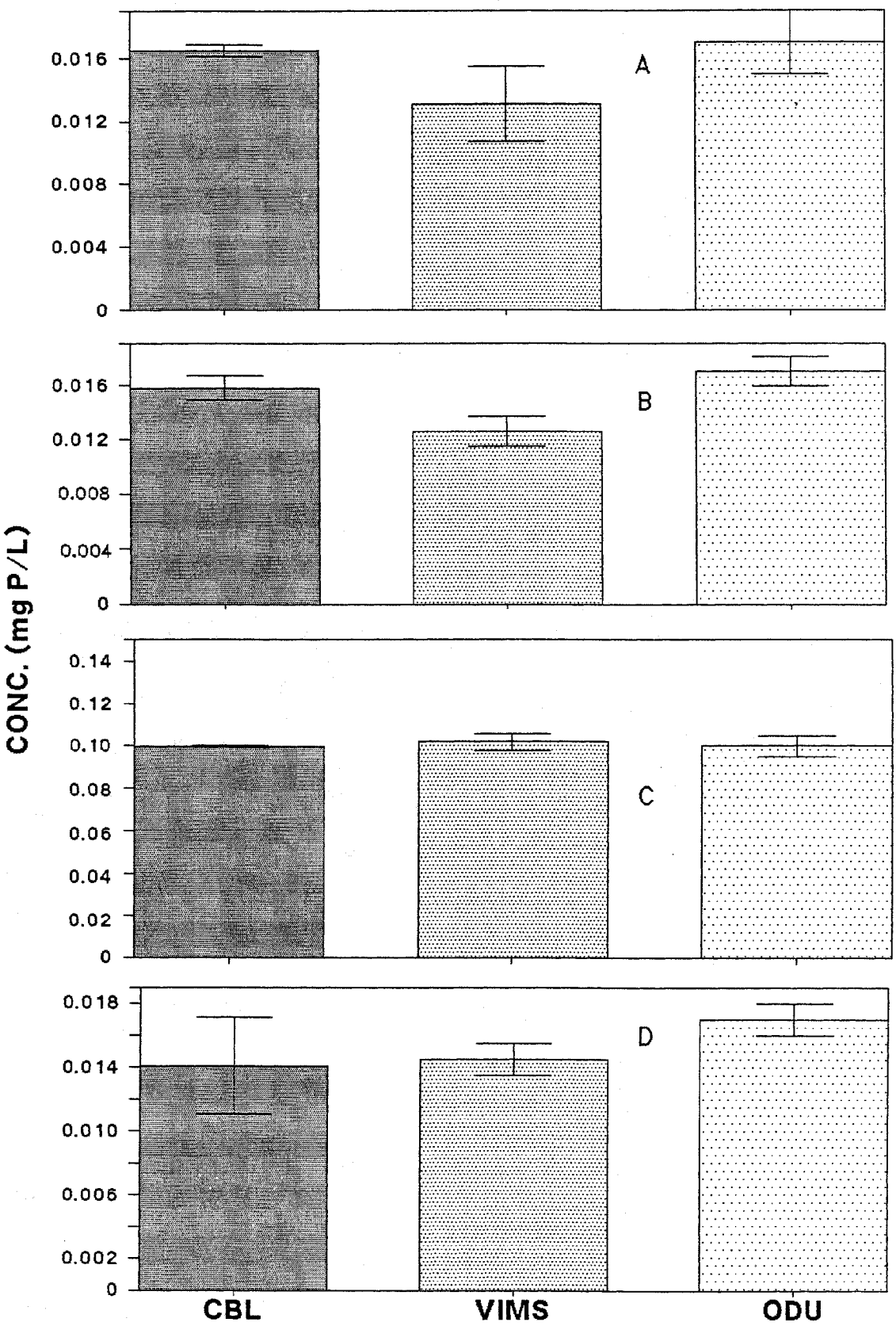


Figure 13. Concentrations of total suspended solids for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

TOTAL SUSPENDED SOLIDS

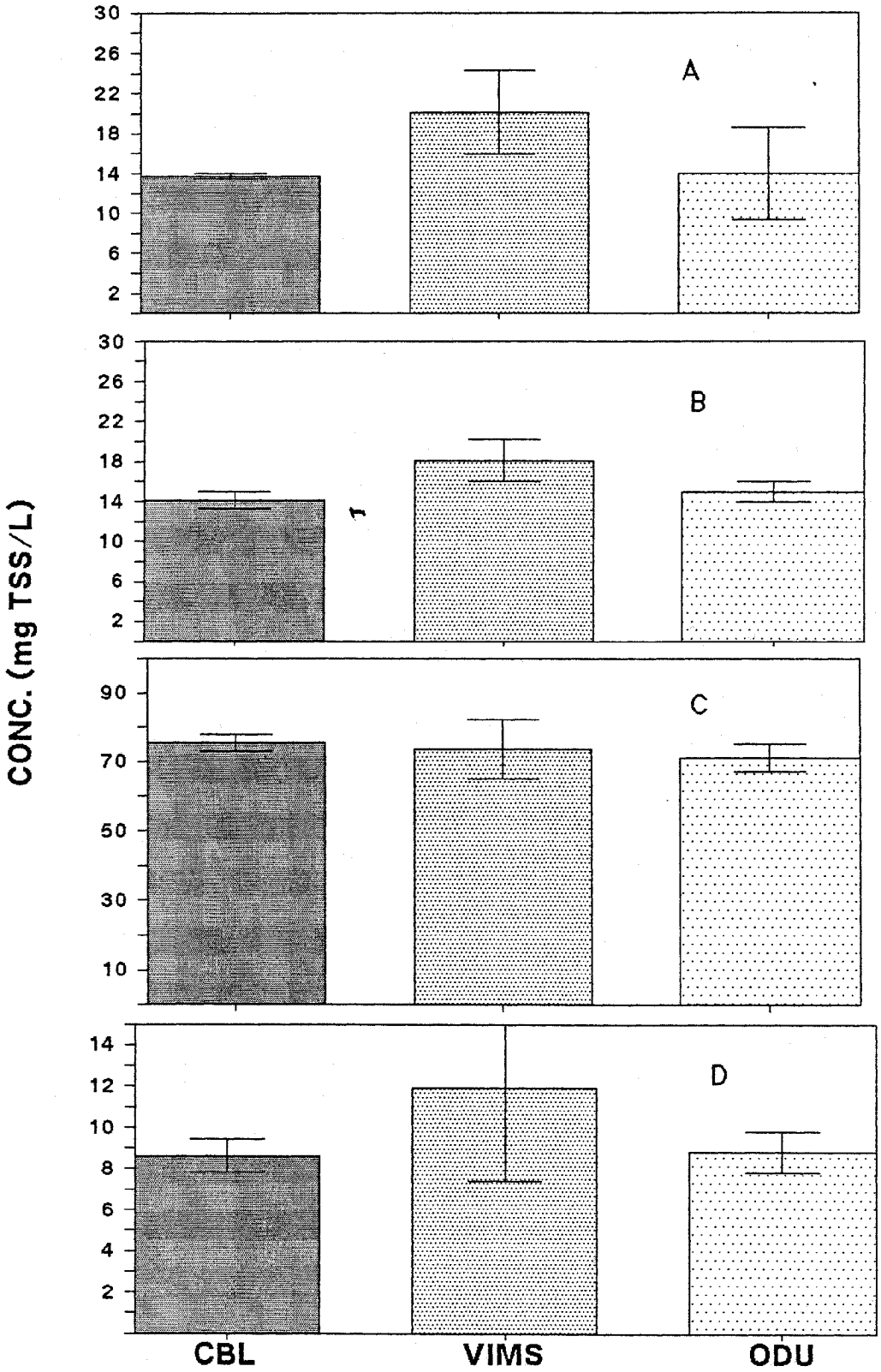


Figure 14. Concentrations of particulate carbon for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

PARTICULATE CARBON

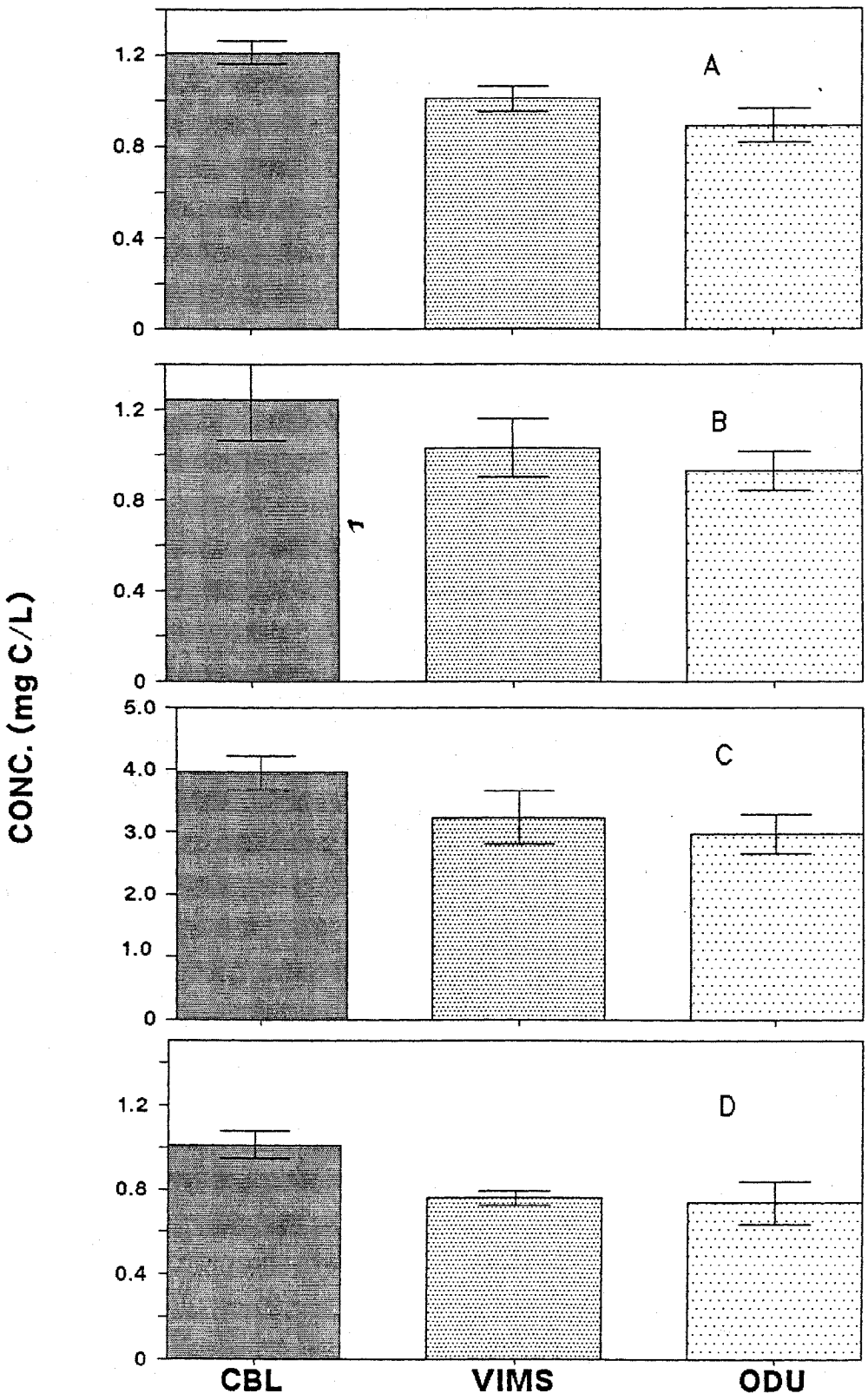
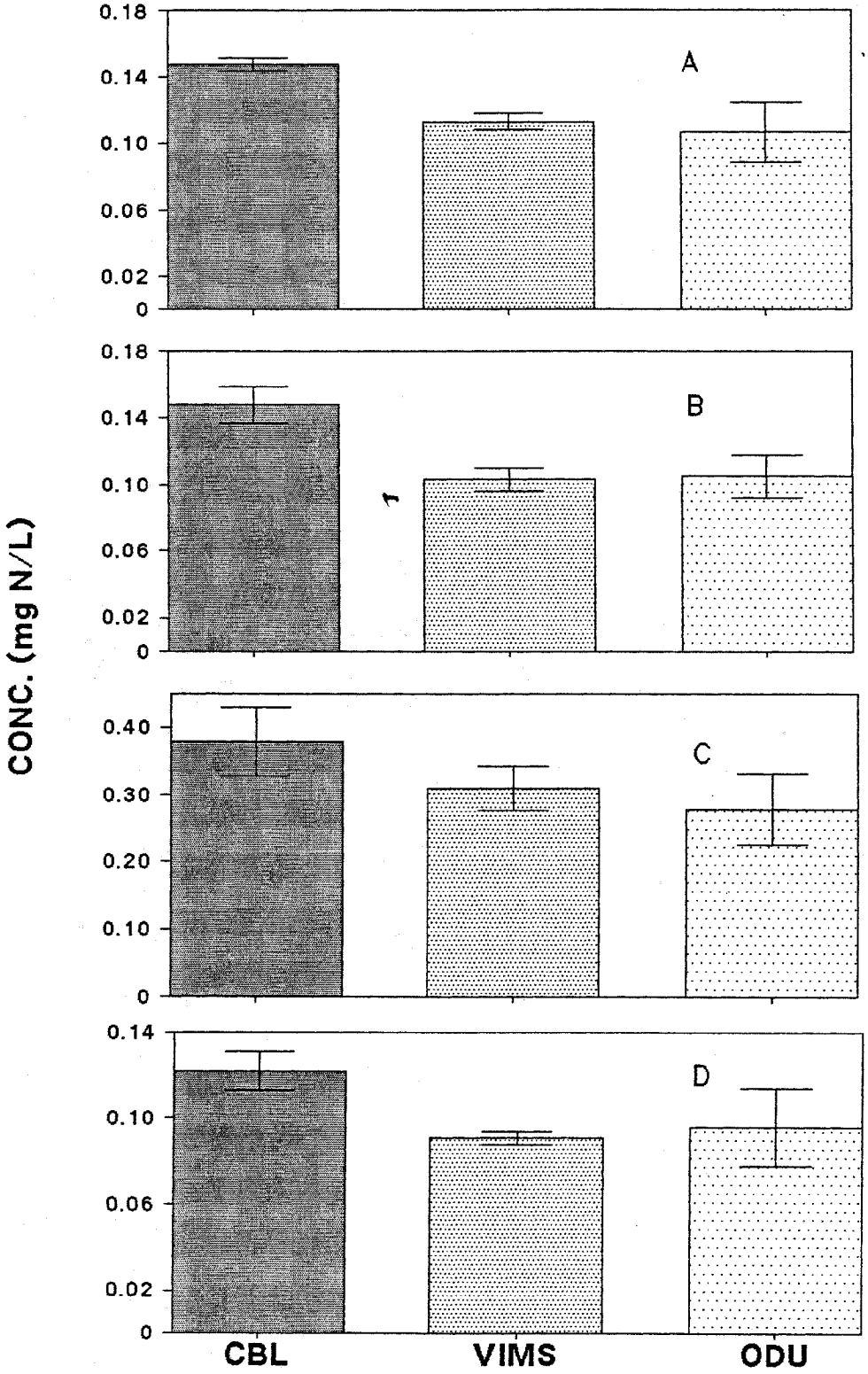


Figure 15. Concentrations of particulate nitrogen for samples A-D determined by Chesapeake Biological Laboratory (CBL), Virginia Institute of Marine Science (VIMS), and Old Dominion University (ODU). Concentration units are mg/L and error bars represent one standard deviation.

PARTICULATE NITROGEN



Particulate C concentrations from CBL were consistently 25% higher than ODU They were also 18 % higher for samples A-C and 25 % higher for sample D than VIMS. Particulate N concentrations reported by CBL were on the average 27% higher than those reported by ODU and 25% higher than those values reported by VIMS.

Two possible explanations exist for these inconsistencies.

Differences in instrument and filter type may be one reason. CBL uses a Control Equipment Model 240 XA Elemental Analyzer which has the capability to analyze 25 mm GF/F filters. VIMS and ODU analyze their samples with a Carlo Erba Model NA 1500 Elemental Analyzer. This instrument can only utilize 13 mm filters. At the time of this study, only 13 mm AE type glass fiber filters were available. Thus, differences in pore size between the two filter types and/or differences in the proportion of catalysts in the instruments may be a possible explanation for these observed differences.

Another possible explanation may have to do with the manner in which the whole water sample is filtered. CBL precombusts the pads, utilizes vacuum filtration and does not rinse the pad with deionized water prior to freezing. ODU precombusts the pads, uses an unregulated form of pressure filtration (Figure 3) and also does not rinse the pad with DI water prior to freezing. VIMS does not precombust the filters, utilizes unregulated vacuum filtration and does post rinse the filters with deionized water (Figure 2). An in-depth study is presently underway to address these procedural differences.

CONCLUSIONS

Inorganic Nutrients: Between laboratory agreement was generally good for nitrite, nitrite+nitrate, ammonium and phosphate. Where the concentrations were low (Samples B and D) the between laboratory variation and among laboratory variation was greatest. Maintaining the integrity of samples that are at or slightly above the detection limit for a specific analyte is very difficult. The possible sources of contamination start with the collection of the sample, continue through the filtering procedure and end with the sample being placed in the instrument for analysis. At each step in this process considerable care needs to be practiced in order to minimize contamination.

In this study, sample processing was performed by experienced personnel under ideal conditions; with the resulting high variability for the low level samples. Imagine then, what degree of variation could be encountered in rough weather on board a rolling research vessel.

Phosphorus: Figure 16 illustrates the total phosphorus (total dissolved + particulate) of the four samples for the three laboratories. Total concentrations are all very similar and the differences in TDP concentrations observed in samples B and D were overshadowed by the high particulate concentrations. Total dissolved P accounted for only 24 and 17% of the total P for samples B and D for CBL, while 39 and 37% of the total was dissolved P for those samples for ODU.

Carbon and Nitrogen: When total carbon (DOC + PC) values for each of the samples are calculated by the three laboratories, the higher particulate values

reported by CBL contribute proportionately more to the total concentrations, so that the total concentrations more closely agree with those of VIMS and ODU (Figure 17).

Sample filtration techniques may then play a role in the observed differences in carbon partitioning. Pressure filtration may lyse cells and consequently more C would become a part of the dissolved fraction which would have otherwise stayed in the particulate phase.

The question of whether or not to rinse the filter pads with deionized water may also contribute to these differences between fractions. A portion of the dissolved sample is retained within the confines of the glass fiber filter. The exact amount may vary. Preliminary results of the differences between PC and PN results of samples which have been rinsed with deionized water versus non-rinsing are significant. For particulate carbon a difference of 0.08 mg C/L was noted, with consistently higher concentrations associated with the non-rinsed pads. The same is true with particulate nitrogen with a mean difference of 0.016 mg N/L between rinsed and non-rinsed pads. If these corrected values are applied to the data for PC/PN for samples A-D, the consistently higher 25 % difference between CBL and the other two laboratories is reduced to 18% for carbon and a mean 18% for nitrogen.

A question often asked by laboratory personnel involved in monitoring efforts is "How much variation are managers/modellers able to accept in their attempts to make sound environmental decisions?"

The study described in this report controlled many of the variables normally associated with a monitoring effort. The samples which were analyzed were from enclosed containers thereby eliminating natural patchiness; the laboratories were experienced in these types of analyses and all used similar analytical techniques. And still, differences occurred.

Figure 16. Total phosphorus (TDP + PP) in mg P/L for four samples analyzed by the three Chesapeake Bay Mainstem Monitoring laboratories. Lower portion of the bar (heavily shaded) is the total dissolved fraction.

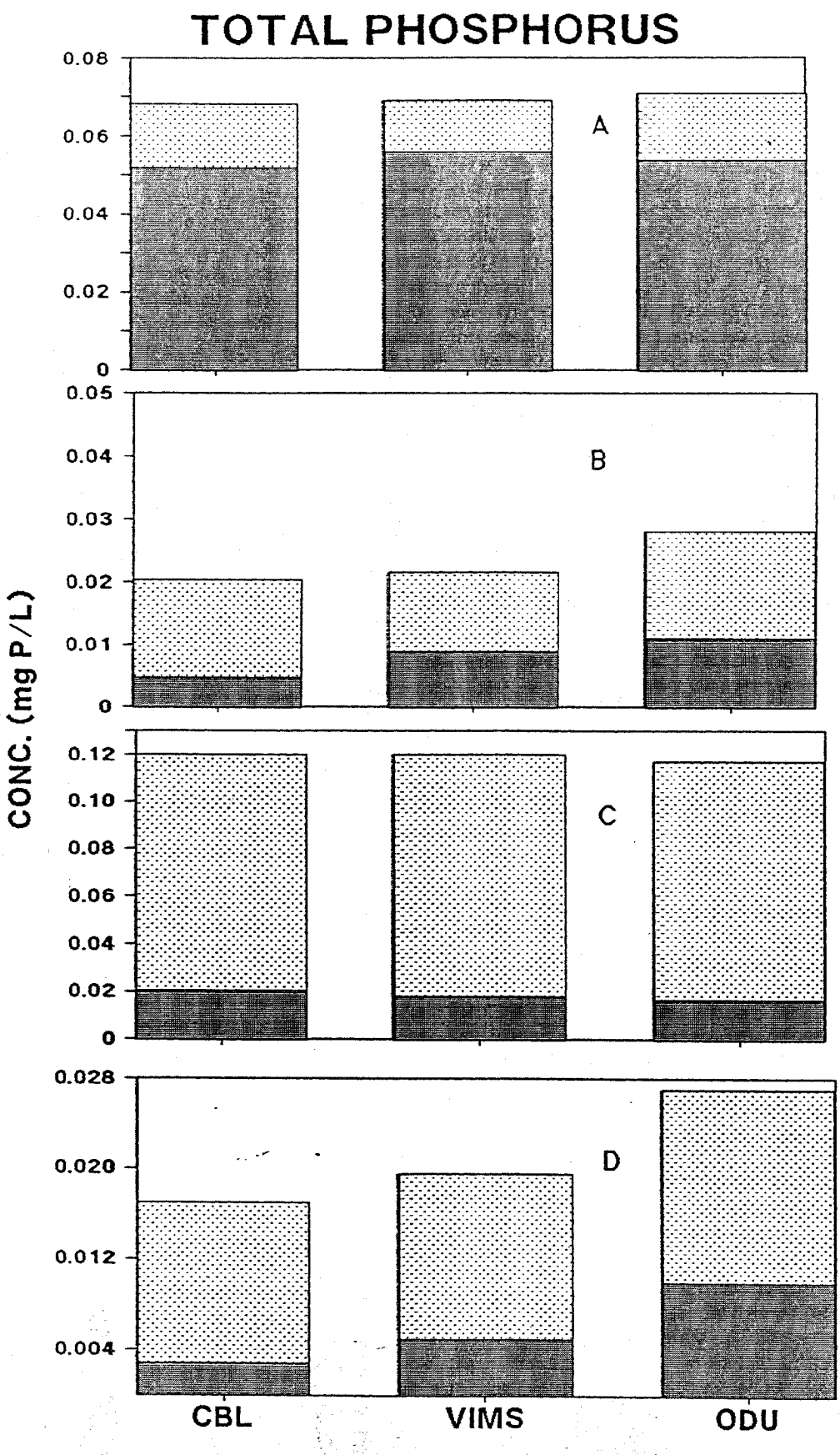

TOTAL PHOSPHORUS
CONC. (mg P/L)
A
B
C
D
0.08
0.06
0.04
0.02
0
0.05
0.04
0.03
0.02
0.01
0
0.12
0.10
0.08
0.06
0.04
0.02
0
0.028
0.020
0.012
0.004
CBL
VIMS
ODU

Figure 17. Total Carbon (DOC + PC) in mg C/L for four samples analyzed by the three Chesapeake Bay Mainstem Monitoring laboratories. Lower portion of the bar (heavily shaded) is the total dissolved fraction.

TOTAL CARBON

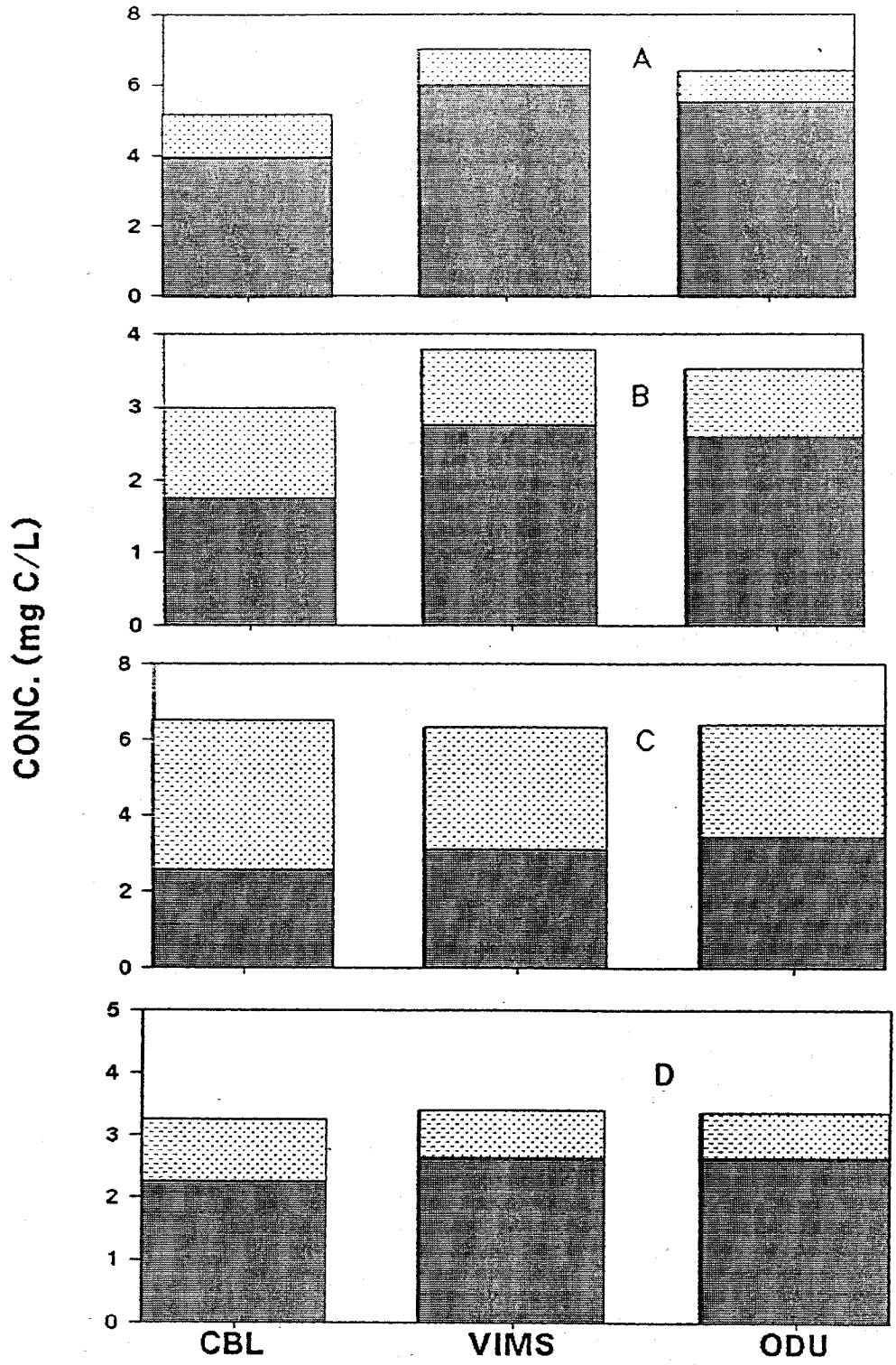
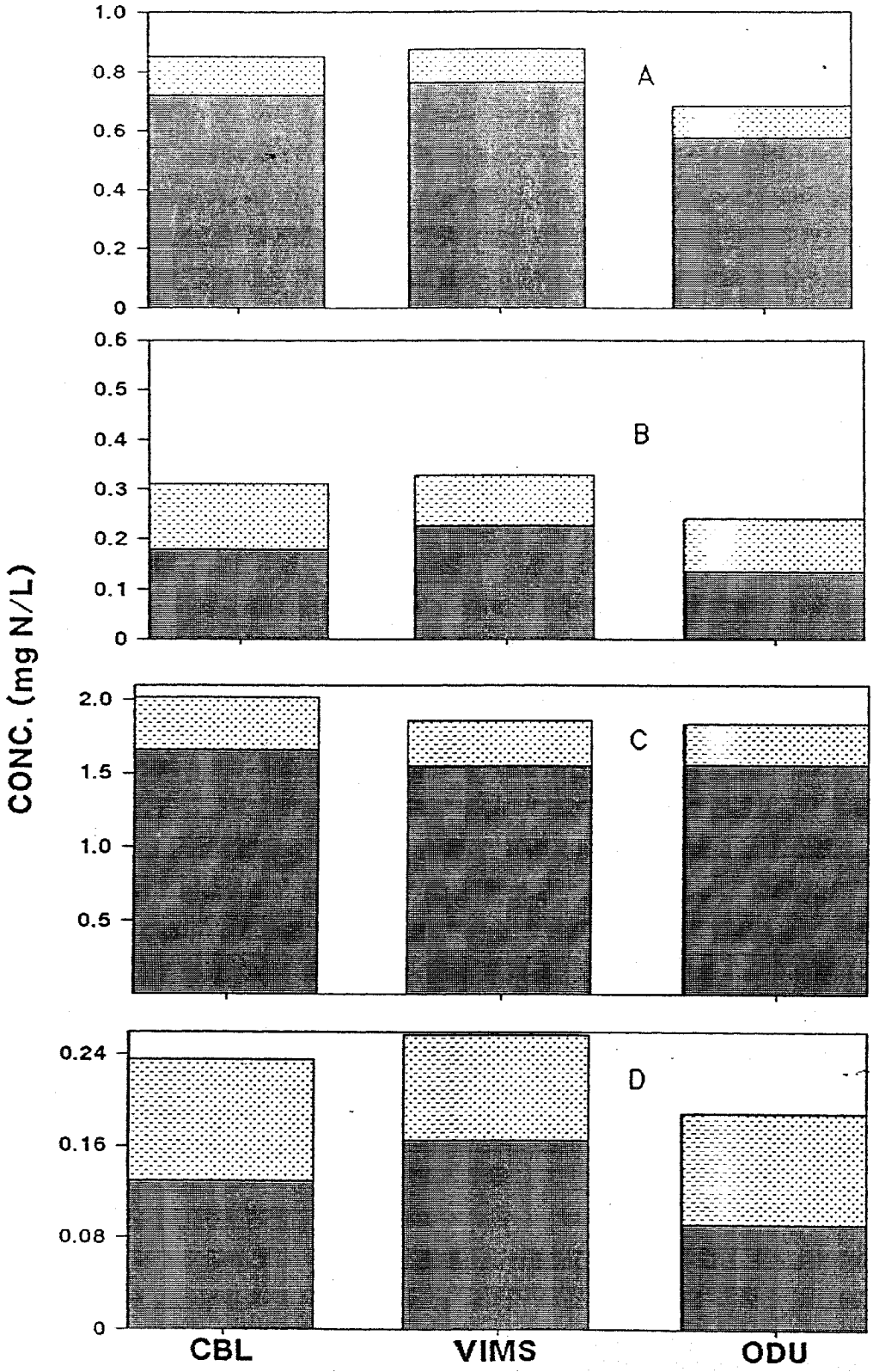


Figure 18. Total nitrogen (TDN + PN) in mg N/L for four samples analyzed by the three Chesapeake Bay Mainstem Monitoring laboratories. Lower portion of the bar (heavily shaded) is the total dissolved fraction.

TOTAL NITROGEN



Agencies involved in the planning or implementation of new monitoring efforts can learn several important lessons from the data presented in this report:

1. Use the best possible sampling techniques and be consistent throughout the study. If more than one laboratory/field crew is involved in the effort, their field sampling techniques should be as similar as practically possible. Changes in sampling protocol need to be documented and made by all involved at the same time.

2. Prior knowledge of the nutrient parameters to be examined and the detection limits required before implementation of the program are strongly recommended. To achieve these goals, use the best analytical methods available.

3. Have the laboratories involved in the monitoring program analyze unknowns in the matrix of interest and at concentrations that are consistent with those found in the natural environment.

4. Plan an effective split sample QA program before implementation of the effort. Such a program can identify and help correct potential problems that may be encountered early in a monitoring effort which involves more than one agency.

5. Where more than one laboratory is involved, variation between results of the analysis of the same sample will occur. Establishment of the degree of acceptable variation is important to determine early on in the program- not only for the managers and modelers; but also for the analysts.

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APPENDIX A

INTERLABORATORY COMPARISON SUMMARY
MARCH 1990
CONCENTRATIONS IN MG/L

NUTRIENT	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
PHOSPHATE	CBL	0.0484	0.0005	0.0033	0.0007	0.0112	0.0002	0.0011	0.0004
	VIMS	0.0441	0.0017	0.0017	0.0016	0.0095	0.001	0.0007	0.0005
	ODU	0.051	0.0016	BDL	0	0.015	0.0018	BDL	0
	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
NITRITE	CBL	0.0439	0.0005	0.0005	0.0001	0.0096	0.0003	0.0009	0.0007
	VIMS	0.0472	0.0007	0.0013	0.0003	0.0113	0.0002	0.0005	0.0002
	ODU	0.0433	0.0002	BDL	0	0.0102	0.0002	BDL	0
	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
NO2+NO3	CBL	0.1491	0.0009	0.0053	0.0012	1.3543	0.0127	0.0064	0.0004
	VIMS	0.154	0.003	0.002	0	1.46	0.042	0.007	0.001
	ODU	0.146	0.0017	0.0025	0.0006	1.357	0.0152	0.0075	0.0008
	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
AMMONIUM	CBL	0.085	0.003	0.003	0	0.147	0.003	0.012	0.0008
	VIMS	0.107	0.003	0.005	0.004	0.131	0.002	0.012	0.005
	ODU	0.089	0.0039	0.0056	0	0.124	0.0009	0.012	0.0031
	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
TDP	CBL	0.052	0.0008	0.005	0.0009	0.020	0.0017	0.003	0.0008
	VIMS	0.056	0.005	0.009	0.003	0.018	0.0002	0.005	0.003
	ODU	0.054	0.0023	0.011	0.0012	0.017	0.0005	0.010	0.0013

	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
TDN	CBL	0.720	0.0320	0.180	0.0240	1.66	0.0150	0.130	0.0240
	VIMS	0.764	0.0310	0.227	0.0180	1.55	0.0300	0.166	0.0120
	ODU	0.578	0.0237	0.138	0.0206	1.56	0.0160	0.093	0.0177

	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
PARTIC. P	CBL	0.0165	0.0004	0.0158	0.0009	0.0997	0.0006	0.0141	0.0026
	VIMS	0.0131	0.0024	0.0126	0.0011	0.1020	0.0040	0.0145	0.0010
	ODU	0.0170	0.0020	0.0170	0.0011	0.1000	0.0047	0.0170	0.0009

	SAMPLE	A			B			C			D	
		MEAN	STD.DEV.	corrected	MEAN	STD. DEV.	corrected	MEAN	STD.DEV.	corrected	MEAN	STD. DEV.
PARTIC. C	CBL	1.210	0.049	1.13	1.240	0.179	1.16	3.950	0.269	3.87	1.010	0.063
	VIMS	1.007	0.054		1.030	0.128		3.230	0.430		0.762	0.034
	ODU	0.890	0.073		0.930	0.084		2.960	0.309		0.740	0.100

	SAMPLE	A			B			C			D	
		MEAN	STD.DEV.	corrected	MEAN	STD. DEV.	corrected	MEAN	STD.DEV.	corrected	MEAN	STD. DEV.
PARTIC. N	CBL	0.147	0.004	0.131	0.148	0.011	0.132	0.379	0.051	0.363	0.122	0.009
	VIMS	0.113	0.005	0.113	0.103	0.007	0.103	0.309	0.033	0.309	0.091	0.003
	ODU	0.107	0.0179	0.107	0.105	0.0133	0.105	0.278	0.0522	0.278	0.096	0.018

	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
TSS	CBL	13.7	0.315	14.1	0.886	75.4	2.61	8.6	0.79
	VIMS	20.1	4.2	18.1	2.1	73.6	8.6	11.9	4.5
	ODU	14	4.6	15	1.01	71.1	4	8.8	0.98

	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
CHLOROPHYLL	DHMH	4.93	0.41	4.74	0.27	5.12	0.80	7.26	0.51
	VIMS	4.70	0.27	4.47	0.25	5.98	1.38	7.72	0.80
	ODU	6.10	1.32	5.50	0.97	3.60	1.26	8.80	1.12

	SAMPLE	A		B		C		D	
		MEAN	STD.DEV.	MEAN	STD. DEV.	MEAN	STD.DEV.	MEAN	STD. DEV.
DOC	CBL	3.96	0.159	1.76	0.082	2.56	0.141	2.26	0.197
	VIMS	6.01	0.210	2.76	0.150	3.11	0.370	2.65	0.120
	ODU	5.52	0.194	2.60	0.134	3.43	0.509	2.64	0.174